

NOTES

THE MONOTROPOIDEAE IS A MONOPHYLETIC SISTER GROUP TO THE ARBUTOIDEAE (ERICACEAE): A MOLECULAR TEST OF COPELAND'S HYPOTHESIS. Kenneth W. Cullings, Department of Biology, San Francisco State University, San Francisco, CA 94132 and Lena Hileman, Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138.

The Monotropoideae (Ericaceae) is a group of plants, mostly California natives, with an achlorophyllous, mycotrophic habit; all plants in this subfamily depend upon shared mycorrhizal connections with neighboring photosynthetic plants for carbon nutrition (Björkman, *Physiologia Plantarum* 13:309–327, 1960). These associations can be quite specific; the most specific mycorrhizal association of any kind is between *Pterospora andromedea* and the mycorrhizal fungus *Rhizopogon* (Cullings et al., *Nature* 379:63–65). The morphology and physiology of these symbioses can be defining characters in the taxonomy of the Monotropoideae and related subfamilies of the Ericaceae (Cullings, *Journal of Evolutionary Biology* 7:501–516, 1994; Cullings et al., *Nature* 379:63–65, 1996), and detailed molecular analysis of relationships among members of the Monotropoideae with respect to these characters has been described (Cullings, *Journal of Evolutionary Biology* 7:501–516, 1994).

Both morphological (Copeland, *Madroño* 6:97–119, 1941; Wallace, *Bot. Notiser* 128:286–298, 1976) and molecular evidence (Cullings and Bruns, *Canadian Journal of Botany* 70:1703–1708, 1992; Cullings, *Journal of Evolutionary Biology* 7:501–516, 1994) strongly indicate that the bulk of the Monotropoideae share a most recent common ancestor with the Arbutoideae (Ericaceae). However, morphological heterogeneity prompted Copeland (*Madroño* 6:97–119, 1941) to hypothesize that the Monotropoideae may be polyphyletic within the Arbutoideae. Recently, Cullings (*Journal of Evolutionary Biology* 7:501–516, 1994) provided strong molecular evidence that one monotropoid species, *Monotropis ordata*, is in fact most closely related to members of the Vaccinioideae, and that the Monotropoideae is polyphyletic. Furthermore, this study confirmed the close association between the bulk of the Monotropoideae and Arbutoideae. However, as the sampling of members of the Arbutoideae was limited to two *Arctostaphylos* species, Copeland's hypothesis that the Monotropoideae has more than one origin within the Arbutoideae has not been fully addressed using molecular methods.

In this study, partial 28S nuclear ribosomal RNA (nrRNA) gene sequences were used to test Copeland's hypothesis. Sequences were generated using the polymerase chain reaction (PCR) and plant-specific primers developed by Cullings (*Molecular Ecology* 1(4):233–240, 1992). PCR products were sequenced directly using the asymmetric primer ratio method (Gyllenstein and Erlich, *Proceedings of the National Academy of Sciences U.S.A.* 85:7652–7658, 1988) and resulted in 500 bases of easily alignable sequence with 2 small indels that were omitted from the analysis. Data were analyzed cladistically using PAUP vers. 3.1.1 (Swofford, D. L. Computer program distributed by Illinois Natural History Survey, Champaign, III, 1995), and by neighbor-joining using PHYLIP vers. 3.572. All analyses were performed with characters unweighted, and bootstrapping was used to assess strength of clades. Trees were rooted by the outgroup comparison with *Clethra* (Clethraceae, Ericales).

Results of this study indicate that the Monotropoideae and Arbutoideae are monophyletic sister groups (Fig. 1), and thus do not support Copeland's hypothesis that the bulk of the Monotropoideae are polyphyletic within the Arbutoideae. Relationships within the Monotropoideae depicted by parsimony analysis of 28S rRNA data have been reviewed previously, and are supported by both morphological and bio-

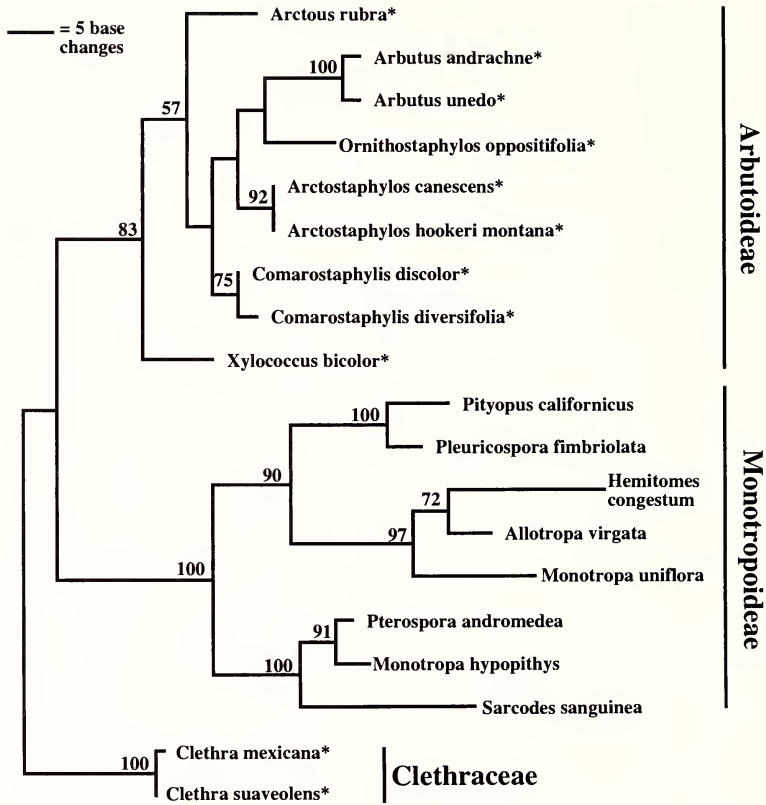


FIG. 1. Phylogeny of the Arbutioideae and Monotropoideae based on partial 28S ribosomal RNA gene sequences. Analysis resulted in a single most parsimonious tree with length: 234, C.I.: 0.709, R.I.: 0.796, rescaled C.I.: 0.565. Numbers at nodes are bootstrap indices of support (1000 replicates). New sequences are indicated by *, all others have been published previously (Cullings, *Journal of Evolutionary Biology* 7: 501–516, 1994). Tree is drawn to scale shown.

chemical data (Cullings, *Journal of Evolutionary Biology* 7:501–516, 1994). Relationships within the Arbutioideae are unresolved by these data (Fig. 1); genera are well supported as monophyletic, but relationships among genera could not be resolved. These relationships are currently being determined using the internal transcribed spacer (ITS), a more rapidly evolving portion of the rRNA repeat unit (Jorgenson and Cluster, *Annals of the Missouri Botanical Garden* 75:1238–1247, 1988).

Taken together, molecular data provide a comprehensive picture of evolutionary patterns within a broadly defined Ericaceae. Both nuclear (28S rRNA) and chloroplast (rbc-L) data indicate that the Arbutioideae split from the Ericaceae relatively early, and that the ericoid mycorrhiza-formers, a group that includes the bulk of the Ericaceae, plus the Empetraceae and Epacridaceae (Kron and Chase, *Annals of the Missouri Botanical Garden* 80(3):735–741, 1993; Cullings, *Canadian Journal of Botany* in press), form a derived group relative to the Arbutioideae. Nuclear data provide further resolution, and indicate that the Ericaceae split into two main groups, the

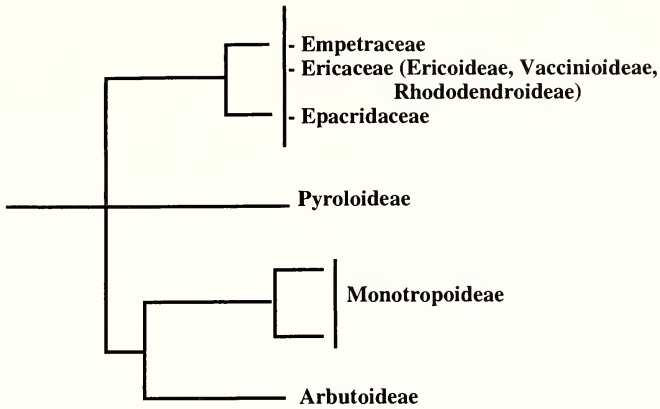


FIG. 2. Schematic of evolutionary trends within the Ericaceae based on a consensus of chloroplast (*rbc-L*) and nuclear (28S rRNA) DNA data. Tree indicates that the arbutoid and monotropoid mycorrhiza-forming groups, Arbutoideae and Monotropoideae, form a monophyletic sister group to the ericoid and pyroloid mycorrhiza-formers, and that the position of the Pyroloideae is unresolved by molecular data.

arbutoid/monotropoid mycorrhizae formers, a clade consisting of the Arbutoideae and Monotropoideae, and the ericoid mycorrhiza-formers (Cullings, *Journal of Evolutionary Biology* 7:501–516, 1994) (Fig. 2). The Pyroloideae, a group that forms mycorrhizae that are intermediate in form, cannot be placed within either clade. Based on this, it appears that one of the pressures influencing cladogenesis within the Ericaceae may have been nutrient access via different mycorrhizal strategies, with the ericoid mycorrhiza clade liberating nutrients from acidic soils (Cullings, *Journal of Evolutionary Biology* 7:501–516, 1994; Specht, *Heathlands and Related Shrublands*, R. L. Specht (ed.), Elsevier Scientific Publishing Company, New York, 1977), and the arbutoid/monotropoid clade receiving carbon through mycorrhizal connections with ectomycorrhizal fungi shared in common with neighboring trees. Members of the Monotropoideae have taken this strategy to the extreme, and have become achlorophyllous and wholly dependent upon these shared mycorrhizal connections (Björkman, *Physiologia Plantarum* 13:309–327, 1960; Cullings et al., *Nature* 379:63–65, 1996). However, because the Monotropoideae are achlorophyllous, it is likely that much of their chloroplast genome has been reduced or lost, as in the case of the achlorophyllous plant parasites in the Orobanchaceae (dePamphilis et al., *Nature* 348: 337–339, 1991). Thus, corroborating data from chloroplast DNA may not be possible with *rbc-L*, though other chloroplast loci may prove useful. At present this possibility is being explored.

LECTOTYPIFICATION OF *SETARIA TENAX* VAR. *ANTRORSA* (GRAMINEAE).—Laurence J. Toolin, Herbarium, Shantz 113, University of Arizona, Tucson, AZ 85721.

Setaria tenax (Rich.) Desv. (*Panicum tenax* Rich.) is a neotropical perennial in the subgenus *Setaria* that ranges from southern Mexico to northern South America. The type (FI; fragment, US!) was collected in Cayenne, French Guiana. Within its range, *S. tenax* is best distinguished from its congeners by the following combination of characters: the awns subtending the spikelets bear both antrorse and retrorse barbs; the spikelets are 2.2–2.5 mm long and somewhat hemispheric; the first glume is one-